Further observations on Chromosomes and Sex-determination in Abraxas grossulariata.

By the late

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The following paper was left by the late Professor Doncaster in an incompleted condition, and, as I was one of his assistants during his last year's work, it has been entrusted to me to prepare for publication.

The paper as it now stands is exactly as Professor Doncaster left it, except that I have added the account of the staining methods used to test the nature of the elimination plate. I was familiar with his staining methods because I was myself testing for chromatin in some entirely different work when Professor Doncaster was testing the elimination plate, and he kindly passed on all his stains to me as he used them, showed me his preparations, and discussed the whole matter with me. In his rough notes I find a full account of all the stains used, and carefully labelled figures showing the results obtained from the different staining methods: there is, therefore, no uncertainty about the facts which I have added.

A summary of the paper included amongst Professor Doncaster's rough notes shows that he intended to add three other sections on 'Conjugation, &c., of polar nuclei', 'Binucleate eggs', and 'Gynandromorphs'. These sections were unfortunately not written even in note form, and therefore cannot possibly be produced; but the paper as it stands is of such obvious interest that its publication even in this very incomplete form is more than justified.

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Further Observations on Chromosomes and Sex-determination in Abraxas grossulariata.

In a previous paper ('Journ. of Genetics', iv, 1914, p. 1) I described the inheritance of a tendency to produce families consisting chiefly or entirely of females in Abraxas grossulariata, and attempted to correlate it with the behaviour of the chromosomes. It was found that females of the strain in which unisexual families occurred have fifty-five chromosomes as the somatic number, while all males and most other females have fifty-six. In the maturation of the fifty-five-chromosome strain, twenty-eight chromosomes travel to one pole of the first polar spindle and twenty-seven to the other. Since all spermatozoa were found to have twenty-eight, it seemed evident that eggs with twenty-seven must be female-determining, since the union of an egg having twenty-seven with a sperm having twenty-eight would give fifty-five, the number found in females of the strain in question, while eggs with twenty-eight meeting sperms with twenty-eight would give the fifty-six found in the male. Evidence was also given that in families in which great excess of females was produced, a majority of the eggs matured in such a way that twenty-eight chromosomes were extruded in the first polar nucleus, and twenty-seven remained in the egg-nucleus, and it was therefore inferred that the condition in some families, in which only females were produced, was caused by the invariable extrusion of the twenty-eighth chromosome in the polar body, leaving all eggs with only twenty-seven, and therefore female-producing. This hypothesis was supported by the observations of Morgan on Phylloxera, in which one chromosome is always extruded in the polar body of male-producing eggs, although it is already determined in some other way that these eggs will become males.

When the paper referred to was published, I had been able to obtain no completely conclusive evidence that in families consisting wholly of females all the eggs had only twenty-seven chromosomes in the egg-nucleus, and I spent the next two seasons in collecting material which it was hoped would give

an unequivocal result. The method adopted was to pair females belonging to all-female families, allow them to lay eggs as far as possible under observation, and to preserve the first 50 or 100 eggs at an age (about two hours) when the maturation-divisions would be in progress. The moths were then allowed to continue laying, the eggs counted, and reared either to the imago or to larvae in which the sex could easily be determined by dissection. Some of these families produced both sexes, others either females only or females in great excess. The preserved eggs of families which proved all-female were then sectioned, and counts made of the chromosomes in the polar division-spindles.

By the summer of 1915 I had already enough material to show that the hypothesis put forward in the 1914 paper was almost certainly incorrect, and since work in connexion with the war prevented the immediate continuation of the investigation I published a preliminary note in a letter to 'Nature' (June 10, 1915) in which I wrote as follows: 'I have now examined the eggs of several such families [i.e. all-female families], and find, contrary to expectation, that the equatorial plate of the inner spindle contains twenty-eight chromosomes about as frequently as twenty-seven. The new material confirms the observation that twenty-seven occur in one spindle and twenty-eight in the other, but it seems to make it certain that the presence of twenty-eight chromosomes in the inner spindle does not necessarily cause the production of a male—at least in the strain which produces all-female families. A possible explanation of the anomaly is that in all-female families a chromosome is eliminated at a later stage, but at present I have no direct evidence for this.'

From that time to the summer of 1919 the work was interrupted, but enough material had been collected to provide the required observations, and examination of the sections confirms the account shortly given in the letter quoted. There are two questions at issue: (1) whether the all-female families are so because all the fertilized eggs are truly female, or whether they arise through non-viability of male zygotes; (2) if all

zygotes in such families are female, whether the egg-nuclei before fertilization contain always twenty-seven chromosomes, or sometimes twenty-eight.

Evidence that All-female Families are not Caused by Non-viability of Male Zygotes.

In the earlier papers a number of families were recorded in which considerably over half the eggs were reared either to imagines or to larvae in which the sex was definitely determinable, but there still seemed some slight chance that all-female families might arise through death of male zygotes at an early age. This, however, seems to be definitely excluded by the results of later experiments, as is shown in Table I, which gives a list of the all-female, or almost exclusively female families in which at least two-thirds of the eggs were reared to larvae or adults of ascertainable sex.

Table I.

Family.	*Number of Eggs.*	*Eggs Hatched.*	*Larvae or Imagines.*	
			♀	♂
1912.1	97	97	66	1
1912.8 (2)	40	31	28	—
1912.29	110	110	77	—
1912.29 B	83	82	66	—
1913.30	77	77	54	2
1914.9	37	not recorded	28	—
1914.16	47	,,	37	—
1914.18	63	,,	58	—
1914.7	14	,,	9	1
1914.28	62	,,	45	—
1916.7	61	46	42	—
1916.9	27	22	22	—
1916.10	62	56	39	4

In view of the fact that in most cases almost all the eggs hatch, and that as soon as the larvae are old enough to be dissected the sex is already clearly distinguishable, these results make it practically indubitable that the all-female families do not arise in consequence of the death of male eggs or larvae. But the matter can be tested in another way,[1] which makes this conclusion doubly sure.

[1] It seems as though a paragraph has been omitted here, but there is no

The evidence just given seems to prove beyond the possibility of reasonable doubt that all zygotes in the all-female families are female, and that these families do not arise by the death of male zygotes. The problem then presents itself whether all eggs of these families before fertilization contain twenty-seven instead of twenty-eight chromosomes. In the letter to 'Nature' referred to I announced that I found evidence that this was not so, and further work has confirmed this conclusion. In 1914 I preserved eggs from four pairings, of which the eggs subsequently laid yielded only females. The data with regard to these families are as follows, excluding the eggs preserved for microscopic examination:

No. of Family.	*No. of Eggs.*	*Eggs Hatched.*	*Males Reared.*	*Females Reared.*
14.9	37	not recorded	0	28
14.22	74	nearly all	0	28
14.28	62	62	0	45
14.29	38	38	1 ?	21

It will be noticed that in families 14.9 and 14.28 over two-thirds of the eggs kept for rearing were reared to imagines (or in 14.28, thirty-six imagines and nine pupae). The eggs of these same families preserved for microscopic examination gave the following chromosome counts in the equatorial plates of the second maturation division.

14.9. In the inner spindle 27, in the outer 28—four cases recorded as 'good'.

In the inner spindle 27, in the outer 28—two cases recorded as 'probable'.

trace of it in the manuscript unless it be the following, which I find on a page of note-paper along with the manuscript: 'Summaries to 1916 show that all-female families are not due to mortality, due to "lethal" or other causes, of male. Apart from such cases as 14.16, and 14.18 (37 and 58 females from 47 and 63 eggs),* the fact that in all-female families in which over 50 per cent. of the eggs are reared to imagines there are twice as many females per cent. of eggs (64·6 per cent.) as compared with percentage of females in bisexual families (32·3 per cent.) proves this.'

* See table given on previous page, 1914.16 and 1914.18.

In the inner spindle 27, outer not countable—one case recorded as ' good '.

In the inner spindle 28, in the outer 27—three cases recorded as ' good '.

In the inner spindle 28, in the outer 27—four cases recorded as ' probable '.

In the inner spindle 28, outer not countable—one case recorded as ' good '.

In the outer spindle 27, inner not countable—one case recorded as ' good '.

Total, seven cases with the inner spindle containing 27, five of these being ' good ' cases in which there is no reasonable doubt as to the number, and nine cases, in which the inner spindle has 28, four of these being ' good ' cases.

14.22. The counts were less satisfactory; they gave three cases in which the inner spindle had 27 or the outer 28, and five in which the inner had 28 or the outer 27, but in only one could both inner and outer be counted with confidence in the same egg; in this egg the inner spindle had 28 and the outer 27.

14.28. In the inner spindle 27, or the outer 28—four cases (three in which both plates could be counted with fair certainty).

In the inner spindle 28, or the outer 27—four cases (one countable in both plates).

14.29. In the inner spindle 27, or the outer 28—three cases, in two of which both inner and outer plates were countable with fair certainty.

In the inner spindle 28, in the outer 27—one case (fairly good).

Although the number of ' good ' counts in which the chromosomes could be counted with confidence in both inner and outer spindles is not large, some of them, especially in family 14.9, are so certain that no doubt can remain that in many eggs of all-female families the inner spindle contains twenty-eight chromosomes, and adding up all counts in the four families we get seventeen cases in which the inner spindle had twenty-

seven (or the outer twenty-eight) and nineteen with the converse arrangement. In the bisexual family 14.35, in which 15 ♂♂ and 19 ♀♀ were reared from forty-six eggs, four eggs were found in which the inner spindle had twenty-seven, the outer twenty-eight chromosomes, and four with the converse arrangement (all ' good ' counts including both spindles of each egg), so it does not appear that the all-female families have twenty-seven in the inner spindle with any greater frequency than in bisexual families of the same stock.

It seems evident from the facts given above that the determination of sex in the fifty-five-chromosome strain of Abraxas grossulariata does not depend on the passage of the odd chromosome to one or other pole of the first polar division. At the same time, since females of this strain have fifty-five chromosomes in their diploid nuclei and males have fifty-six, a chromosome must be eliminated at some stage from those eggs in which twenty-eight travel to the inner pole of the first polar spindle. Attempts to find a chromosome which does not divide in the second maturation division have not been successful, and it seems clear that the elimination does not occur at that stage. Only two possibilities remain: either a chromosome is eliminated at some division after fertilization—presumably the first segmentation division, or the odd chromosome must degenerate so that the twenty-eight chromosomes present in about half the eggs at the inner pole of the first polar spindle are reduced to twenty-seven by the degeneration of one of them. Neither possibility seems likely on general grounds, but there are some facts which make the hypothesis of the degeneration of a chromosome less entirely improbable than would appear at first sight. These will be discussed in the next section. With regard to the hypothesis of the elimination of a chromosome in the first segmentation division, I can only say that I have not succeeded in obtaining figures in which the chromosomes in this division can be accurately counted: in the few segmentation divisions present in my material the chromosomes tend to become aggregated into small groups, apparently of two or three, so that counts give numbers not

much greater than the haploid complement (twenty-eight). Probably mitotic figures embedded deeply in the yolk are fixed less rapidly than the maturation mitoses near the surface of the egg, with the result that observations on the number and behaviour of the chromosomes in the segmentation divisions become untrustworthy.

'Chromatin Elimination' in the Maturation Divisions of the Egg.

In my 1914 paper [1] I mentioned that 'during the first polar division, a mass of granules which stain deeply with iron haematoxylin is left in the equatorial plate as the chromosomes travel to the poles' (fig. 14 of that paper). No further investigation was made at the time on the nature or mode of origin of these granules, but in a paper published almost simultaneously [2] Seiler describes them in considerable detail in the eggs of the moths Phragmatobia fuliginosa, Orggia antiqua, Lymantria monacha, and L. dispar. He gives evidence that these granules are separated from the chromosomes in the early anaphase of the first polar division, and maintains that in favourable cases it is possible to see that each chromosome, as it divides, leaves behind on the equator of the spindle a chromatin mass which for a time at least preserves its identity, so that in sections of a mitosis in anaphase cut at right angles to the axis of the spindle it is possible to see three plates each containing the same number of chromatin bodies similarly arranged—the two anaphase groups of chromosomes and between them an 'elimination plate' consisting of chromosome-like bodies having the same number and arrangement as the chromosomes in the true chromosome plates. Careful search among my preparations—both old ones and new sections made for the purpose—has not revealed the existence of plates with such definite, chromosome-like granules in Abraxas, but in other respects my sections, when stained

[1] Doncaster, L., 'Journ. of Genet.', 1914, p. 1.

[2] Seiler, J., 'Archiv für Zellforschung.', xiii. Band, 2. Heft (p. 159). Leipzig und Berlin, 1914.

with iron haematoxylin, give very nearly the same series of figures as are represented by Seiler. I have a few cases in very early anaphase (just after metaphase) in which each chromosome seems to be leaving behind, as its halves diverge on the spindle, a mass of staining substance (cf. Seiler's figs. 19–22), and in later anaphase there is always an equatorial plate of staining granules lying across the middle of the spindle. Not infrequently these granules are elongated, so as to appear like short threads, and some or all of them seem to lie on or in the spindle fibres. Towards the end of the anaphase they generally form a plate of fine-stained dots, of varying size, and always more numerous than the chromosomes, as if they had become broken up and scattered. During the second division they sometimes become aggregated into a sort of network (cf. Seiler's fig. 35), or they may apparently have become more finely divided and comparatively inconspicuous.

Like Seiler, I find great variation among different polar mitoses in respect of the amount of this eliminated substance. In some spindles there is a dense equatorial mass, staining with iron haematoxylin almost as deeply as the chromosomes around the poles. In others the granules are much less conspicuous, in others again so few and small as hardly to be noticeable. The amount of staining matter in the 'elimination plate' varies in different eggs of the same female, and even in eggs mounted on the same slide, though on the whole it is more abundant in the eggs of some females than in those of others. It is important to notice, however, that the apparent amount varies with depth of staining, and when sections of several eggs are mounted together on a slide it may happen that some spindles are fully washed out, so that the chromosomes alone remain clearly stained, while a spindle in a neighbouring egg may retain so much stain as to be useless for the study of chromosomes. This variability probably arises from differences of fixation due to variation in the penetrability of the egg-shells to the fixative, and therefore it is not impossible that the variation in the apparent amount of eliminated sub-

stance may be in part at least due to the technique of fixing and staining.

Seiler has no doubt that the stained matter in the elimination plate is chromatin, and reviews the literature of maturation divisions of insect eggs, and also such examples of chromatin elimination as those seen in the segmentation of Ascaris and Miastor, in order to discuss the significance of the process. He does not, however, discuss at all fully the question whether the substance eliminated is in fact chromatin, or if it is, whether it is of the same nature as the chromatin of the anaphase chromosomes. His account of his staining methods is meagre—'Gefärbt wurde vorwiegend mit Heidenhains Eisenhämatoxylin und Kontrollfärbungen wurden mit Kernfarbstoffen vorgenommen. Als Plasmafarbstoff verwendete ich S.-Fuchsin'. Unless the elimination process is in reality an artefact, which seems very unlikely in view of the almost invariable presence of staining granules in the equator of the spindle and the definite manner in which they appear to be left behind by the diverging chromosomes, it appears to be of considerable importance to determine the true nature of the eliminated substance, for if it be chromatin, it seems not impossible that the process may supply the clue to the anomaly presented by the presence of twenty-eight chromosomes in eggs which nevertheless yield females. If the chromosomes do in fact leave behind on the polar spindle a considerable part of their substance, it is at least conceivable that the sex-chromosome, in the eggs of all-female broods, eliminates so much that it becomes functionless as regards sex-determination, and that, having lost so large a part of its substance, it ceases to function and disappears, so that in the oogonia only fifty-five instead of fifty-six can be counted. With the object of determining whether the elimination plate does or does not consist of chromatin I stained eggs with a number of combinations of stains, the more important of which were as follows:[1]

[All the sections used had been previously stained with iron haematoxylin and were decolourized with acid alcohol.]

[1] Professor Doncaster's manuscript ends here.

I. Ehrlich's Triacid Stain.

Sections were immersed for eighteen hours in the stain, blotted, and passed through absolute alcohol and xylol into balsam. The chromosomes and the elimination plate were stained purple, the surrounding protoplasm brown.

II. Safranin and Lightgreen.

Sections were placed in safranin for from twelve to twenty-four hours, followed by lightgreen for one to two minutes. The chromosomes were stained a bright red, the elimination plate a lighter red in some cases and in others green with a distinct admixture of red. The spindle-fibres were green.

Both the above methods gave very clear results which strongly suggest that chromatin was present in the elimination plate.

III. Mann's Methyl Blue Eosin.

Sections were stained for a few minutes only. This method gave very erratic results. In most cases the spindle-fibres were blue, but whereas in some sections the chromosomes and elimination plate were also blue, in others the chromosomes were purple and the elimination plate red ; and in others again the chromosomes were red with a bluish tint here and there, and the elimination plate purple.

In spite of the varying results obtained with this stain it is clear that in any given section there is a very close correspondence between the chromosomes and the elimination plate.

IV. Ehrlich's Haematoxylin.

Sections were stained for eighteen hours with Ehrlich's haematoxylin, differentiated for from one to two minutes in acid alcohol, washed in 70 per cent. alcohol and counterstained with eosin in 90 per cent. alcohol for about one minute. The spindle-fibres always stained pink, the chromosomes were usually black, and the elimination plate pink with grey dots ; sometimes, however, the chromosomes were a bright pink and the elimination plate a paler pink, and at other times the chromosomes and the elimination plate were purple.

Here again, as with Mann's methyl blue eosin, the chromo-

somes and the elimination plate in any given section correspond very closely in their staining properties, although in different sections very different results were obtained from the same combination of stains.

V. Borax Carmine and Picro-indigo-carmine.

Sections were stained in borax carmine for forty-eight hours, followed by picro-indigo-carmine for ten minutes.

The chromosomes were found to be dark red, and the elimination plate and spindle-fibres yellowish. Although this method did not appear to give support to the view that chromatin is present in the elimination plate, it does not disprove that hypothesis, for in some sections even the chromosomes themselves were barely stained with the carmine, so that it is not surprising to find the elimination plate unstained even though it may contain chromatin, for this eliminated chromatin would almost certainly be undergoing disintegration. In sections stained by other methods as given above, it was not unusual to find that the elimination plate was unstained, even though it had previously stained deeply with iron haematoxylin.

These staining experiments, although not conclusive, give a considerable weight of evidence in support of the hypothesis that there is a certain amount of chromatin left behind, on the equator of the spindle, by the chromosomes when they move apart at anaphase. If this be true ' it is at least conceivable that the sex-chromosome, in the eggs of all-female broods, eliminates so much that it becomes functionless as regards sex-determination ',[1] and that here may lie the explanation of the production of all-female families from eggs some of which contain twenty-seven and others twenty-eight chromosomes.

From conversation with Professor Doncaster, as well as from his own argument in this paper, I know that this was the conclusion at which he himself had arrived.

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[1] p. 406.